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A COMPARISON OF THE EFFECTS OF NA_2SO_4 AND NACL ON THE GROWTH OF *HELIANTHUS PARADOXUS* AND *HELIANTHUS ANNUUS* (ASTERACEAE)

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ABSTRACT

Helianthus paradoxus Heiser (Asteraceae, puzzle sunflower), is a federally threatened hybrid species found in salt marshes of west Texas and New Mexico. *Helianthus annuus* L. (Asteraceae, common sunflower) is one of the parent species and is found throughout North America, but it is not present in the inland salt marshes where *H. paradoxus* is found. *Helianthus paradoxus* has previously been described as a halophyte, but its tolerance to Na_2SO_4, one of the major salts found in its habitat, has not been investigated. However, salinity has been identified as a major abiotic factor influencing the limited distribution of *H. paradoxus* populations. In this greenhouse study, the effects of elevated concentrations of Na_2SO_4 and NaCl, at equal ionic strengths (0.00, 0.09, 0.17, 0.34, and 0.51), on the survival and dry mass of both *H. paradoxus* and *H. annuus* were examined. In the three-way factorial experiment, the effects on dry mass observed were dependent on the species, the type of salt and the ionic strength of the salt. *Helianthus paradoxus* produced more dry mass than *H. annuus* in both salt treatments; however, NaCl was more inhibitory of dry mass production for both species with plants unable to survive the highest salt treatments. While dry mass of *H. annuus* decreased with increasing ionic strengths of both salts, dry mass of *H. paradoxus* increased by 38 to 72% in low to moderate ionic strengths of Na_2SO_4 relative to the nonsaline treatment. Both species were less tolerant of NaCl than Na_2SO_4 with *H. paradoxus* seeming to have moderate and high tolerance to elevated Cl^- and SO_4^{2-} ionic strength, respectively, while *H. annuus* had low to moderate tolerance. Greater dry mass production in Na_2SO_4, along with tolerance to both salts, suggests that low to moderate sulfate soil salinity will enhance the dry mass production of *H. paradoxus*.

Key Words: Halophyte, *helianthus annuus*, *helianthus paradoxus*, ionic strength, NaCl, Na_2SO_4, salt tolerance, sunflower.

Helianthus paradoxus Heiser (Asteraceae, puzzle sunflower) is a federally threatened species with limited distribution in salt marshes in west Texas and New Mexico (Correll and Johnston 1979; Poole and Diamond 1993; McDonald 1999). Hybridization studies (Heiser 1958, 1965; Abbott 1992) and molecular analysis (Rieseberg et al. 1990; Rieseberg 1991; Rieseberg et al. 1991) have determined that *H. paradoxus* is a stabilized hybrid species between *H. annuus* L. (Asteraceae, common sunflower) and *H. petiolaris* Nutt. (plains sunflower). Although *H. paradoxus* shares several morphological and ecophysiological traits with its parental species (Rosenthal et al. 2002), it has diverged and is genetically isolated from its progenitors and considered a separate species. *Helianthus anuus* is common throughout North America and grows in disturbed, heavy clay soils that are moist in the spring and dry out by midsummer. *Helianthus petiolaris* is found in dry, sandy soils in western North America, while *H. paradoxus* grows in heavy, waterlogged, saline soils (Van Auken and Bush 1998).

Ecological and ecophysiological studies of *H. paradoxus* have determined that this homoploid hybrid species is salt tolerant, unlike its parental species. *Helianthus paradoxus* is restricted to inland salt marshes with salt levels of approximately 10 g kg^{-1} (Poole and Diamond 1993; U.S. Fish and Wildlife Service 2005; Grunstra and Van Auken 2007a, b), while *H. annuus* and *H. petiolaris* can be found in low saline soils (<0.02 g kg^{-1} soil sodium, Welch and Rieseberg 2002). *Helianthus paradoxus* is a better competitor than its progenitors in saline soils (Bush and Van Auken 2004). The west Texas and New Mexico salt marshes were key habitats in isolating hybrids (Abbott 1992). The parental species are glycophytes and cannot survive the same habitat as *H. paradoxus,* where other salt tolerant plants are generally present (Poole and Diamond 1993; Lexer et al. 2003; U.S. Fish and Wildlife Service 2005). Additionally, *H. paradoxus* is capable of sequestering higher sodium and sulfur concentrations and produces greater leaf succulence compared to its parental species while maintaining significantly greater fitness when grown in elevated NaCl concentrations (Welch and Rieseberg 2002) or in field-like highly saline soil conditions (Karrenberg et al. 2006).

In the largest known population of *H. paradoxus*, at the Diamond-Y Spring Preserve near

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Fort Stockton, Texas, the distribution of *H. paradoxus* is mainly affected by soil salinity and soil moisture gradients when biotic factors are not considered (Bush and Van Auken 1997; Van Auken and Bush 1998; Bush 2006a, b). This large desert spring and associated salt marsh has had 1.44 to 2.70 million *H. paradoxus* plants (Van Auken and Bush 1998), depending on environmental conditions. In addition, there are several rare and federally endangered invertebrates found in the marsh (McDonald 1999). *Helianthus paradoxus* plants consistently establish parallel to the drainage of Leon Creek but their proximity depends on seasonal climatic conditions influencing soil salinity levels and soil water content, both of which decrease dramatically upland from the lowest point in the salt marsh (Van Auken and Bush 1998; Grunstra and Van Auken 2007a, b). When annual rainfall is high, *H. paradoxus* can be found further from the drainage compared to drier years when plants are located closer to the drainage. The population of *H. paradoxus* at the Diamond-Y Spring Preserve seems to be dependent on low to intermediate salinity levels and intermediate moisture levels; however, salinity appears to be the major abiotic factor affecting the local distribution of *H. paradoxus* (Bush 2006a, b).

Previous studies have identified *H. paradoxus* as a salt tolerant species with characteristics of halophytes (Welch and Rieseberg 2002; Bush and Van Auken 2004). However, these studies were done with NaCl, one of the major salt components of the marsh, but not Na_2SO_4 which is also found at high concentrations in soils of its salt marsh habitat (Boghici 1997). In addition, the effects of a limited range of salinity levels have been examined. Chloride is generally more toxic than SO_4^{2-} and even Na^+ at lower concentrations (Manchanda et al. 1982; Marschner 1995; Franklin and Zwiazek 2004; Munns and Tester 2008); however, SO_4^{2-} salinity can be more growth inhibitive than Cl^- for some halophytes (Warne et al. 1990; Egan and Ungar 1998). At the same time, separating specific ion effects is difficult and differential effects of Cl^- and SO_4^{2-} salinity on *H. paradoxus* are still unknown; consequently, the influence of these anions on the ecological isolation and distribution of *H. paradoxus* has not yet been elucidated.

Ionic strength rather than salt concentration was used as a treatment variable in the present study. It is used as a normalization procedure and was required because of different numbers of ions present in equal molar solutions of NaCl (2 ions) and Na_2SO_4 (3 ions). Therefore, it was important to compare the two salts at concentrations that allowed osmotic potential of the corresponding treatments to be equal. Concentrations of the two salt treatments were based on equal ionic strengths calculated using procedures in Barrow (1966).

TABLE 1. IONIC STRENGTHS OF EACH TREATMENT AND CORRESPONDING SALT CONCENTRATIONS IN $g \cdot kg^{-1}$ EXAMINED IN THE STUDY.

Ionic strength	NaCl (g kg^{-1})	Na_2SO_4 (g kg^{-1})
0.00	0	0
0.09	5	4.1
0.17	10	8.1
0.34	20	16.2
0.51	30	24.3

The objective of this study was to examine the survival, growth and the salt tolerance of *H. paradoxus* in elevated levels of both Na_2SO_4 and NaCl, at equal ionic strengths. Both of these salts are major contributors of the soil salts of the Diamond-Y Spring Preserve. Our experiment also included the more salt tolerant of its parental species, *H. annuus*, a known glycophyte.

MATERIALS AND METHODS

Helianthus paradoxus seeds were collected from the Nature Conservancy's Diamond-Y Spring Preserve near Fort Stockton, Texas (31°00.54′N, 102°55.49′W) and stored dry at 25°C until used. *Helianthus annuus* seeds were purchased from Native American Seed Farm (Junction, Texas 76849) and stored dry at 4°C. Seeds of both species were cold stratified in Ziploc® storage bags lined with paper towels, wet with deionized water, and maintained at 4°C in the dark for 4 weeks (*H. paradoxus*) or 8 weeks (*H. annuus*). To prevent osmotic shock, groups of seeds (approximately 400 seeds for each treatment) were germinated on paper toweling saturated with deionized water (0.00 osmotic strength) in plastic storage bags at 25°C. Germinated seedlings were transferred to equal or increasing levels of the appropriate salt (NaCl and Na_2SO_4 at ionic strengths of 0.00, 0.05, 0.09, 0.13, 0.17, 0.26, 0.34, 0.43, and 0.51) every 2 d until placed in the final salt (NaCl or Na_2SO_4) and ionic strength to be tested (0.00, 0.09, 0.17, 0.34, and 0.51). Seedlings were kept in the appropriate solution for a total of 18 days prior to transplanting to pots in the greenhouse containing the corresponding treatment (Table 1).

For each species, five plants per pot with five replicate pots per treatment (25 plants per species per treatment) were grown for 62 d. Plastic pots (15 cm diameter × 15 cm height) lined with a Ziploc® storage bag (to retain water, salts, and nutrients) were filled with 1.4 kg of air-dried, sieved (5.8 mm mesh screen) soil. The soil was the upper 10 cm of a low nutrient Patrick-series Mollisol (clayey-over-sandy, carbonatic-thermic, typic calciustoll), collected from northern Bexar Co., Texas (Taylor et al. 1966; Van Auken and Bush 1998). The soil was friable, allowing root

TABLE 2. THREE-WAY ANOVA RESULTS FOR THE TOTAL DRY MASS (G) OF TWO SPECIES (*HELIANTHUS PARADOXUS* AND *H. ANNUUS*) GROWN IN TWO SALTS (NACL AND NA_2SO_4) AND AT FIVE IONIC STRENGTHS (0.00, 0.09, 0.17, 0.34, AND 0.51). All main effects and interactions were significant. [a] Significant interactions are designated (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$).

Source[a]	df	SS	F	P
Species	1	4.11	35.69	<0.0001
Salt	1	14.75	128.17	<0.0001
Strength	4	5.00	43.44	<0.0001
Species × salt***	1	1.39	12.05	0.0008
Species × strength**	4	0.55	4.78	0.0016
Salt × strength****	4	1.67	14.50	<0.0001
Species × salt × strength*	4	0.40	3.51	0.0108
Error	80	9.21		
Total	99	59.95		

extraction and recovery (Bush and Van Auken 2004). Appropriate amounts of anhydrous salts (Na_2SO_4 or NaCl) and a single nutrient application (0.05 g of P from $Na_2HPO_4 \cdot 7H_2O$, 0.07 g N from NH_4NO_3, 0.07 g of K from KCl, and 0.03 g of S from $MgSO_4 \cdot 7H_2O$; Tiedemann and Klemmedson 1986) were added to each pot and thoroughly mixed. Before seedlings were planted, 400 ml of deionized water was added to each pot. Thereafter, soil moisture was maintained at approximately field capacity with distilled water. This study was conducted in a fiberglass greenhouse in which the daytime temperatures ranged from approximately 26° to 38°C and light levels were approximately 36% of full sunlight with a mean photosynthetically active photon flux densities of 562 ± 135 $\mu mol\ m^{-2}\ s^{-1}$ measured with a Li-Cor® Ll-188 integrating quantum sensor.

Plant survivorship was assessed at 62 d for each treatment combination (species × salt × ionic strength). Percent survivorship is based on the mean number of erect and green plants out of five plants per replicate pot per treatment.

Shoot and root dry mass were measured at the end of the experiment. For shoot dry mass, plants from each pot were clipped at the soil surface and placed in a pre-weighed paper bag. For the ash-free root dry mass, all of the soil and particulate matter from each pot was carefully washed off the roots. Roots were then wrapped in pre-weighed aluminum foil. All plant material was dried in a forced air oven at 90°C to a constant mass. After drying, roots were ashed in a programmable muffle furnace (Fisher Scientific Isotemp®, Fisher Scientific Research, Pittsburgh, PA) at 625°C for 3 hours to obtain the ash-free root dry mass (Böhm 1979). Total dry mass was also calculated by combining the shoot dry mass and ash-free root dry mass per pot.

All dry mass data was analyzed using SAS statistical software (SAS Institute 1999) with each pot as the unit of replication and $P \leq 0.05$ as the criterion for significance. Any plant mortalities because of a treatment effect were taken into account by including a dry mass of zero. To determine the effects of salt type and ionic strength on the dry mass of each species, a three-way ANOVA including interactions was employed. When significance was detected with the overall ANOVA, two-way and one-way ANOVAs were used followed by Duncan's Multiple Range Test to examine significant differences between all possible combinations of salt type and salt concentration for each species separately. Mean mortality was calculated, but was not analyzed statistically.

RESULTS

All *H. paradoxus* and *H. annuus* plants died in the highest ionic strengths of NaCl examined (0.34 and 0.51). However, both species demonstrated 100% survivorship in the no-salt treatment. For all Na_2SO_4 treatments, 100% of *H. paradoxus* plants survived, while 100% of the *H. annuus* plants survived in each of the Na_2SO_4 treatments except at the 0.51 ionic strength, the highest Na_2SO_4 concentration tested. Plant survivorship for *H. paradoxus* in NaCl treatments was 100% at the 0.09 and 0.17 ionic strengths, while 80% and 40% of *H. annuus* plants survived in these same ionic strengths, respectively.

Three three-way ANOVAs were used to analyze shoot, root, and total dry mass; however, only total dry mass data will be presented. Results were similar for mean shoot and root dry mass (analysis not shown). The three-way ANOVA (Table 2) demonstrated a significant overall species, salt, and ionic strength effect on total dry mass. In addition, the three two-way interactions were significant as was the three-way interaction. To demonstrate more clearly the experimental results, two of the two-way interactions will be presented first. Overall, *H. paradoxus* produced more dry mass than *H. annuus* in both salts (Fig. 1a, species × salt interaction). For both species, more dry mass was produced in the Na_2SO_4 treatment than in the NaCl treatment. In addition, ionic strength was significant with more dry mass in the lower treatments;

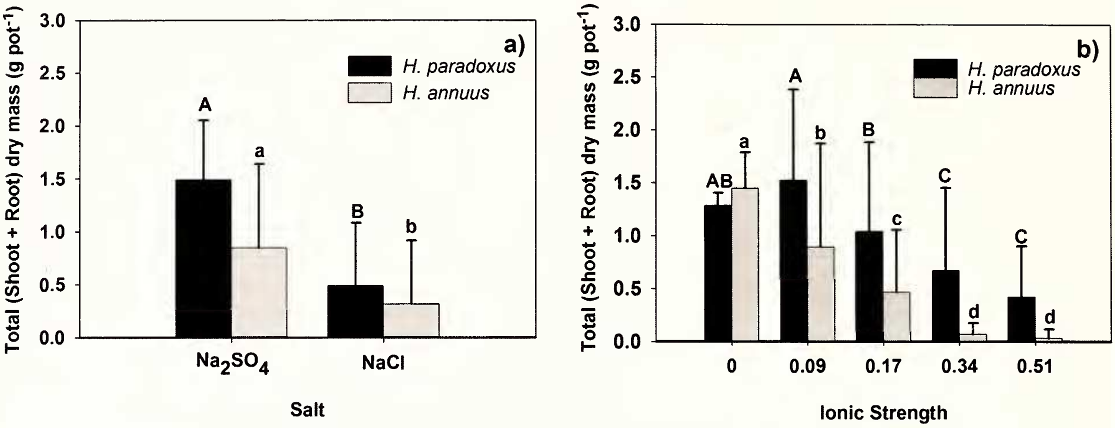

FIG. 1. Two-way interaction plots of total dry mass (bars) as the response variable for (a) salt (NaCl and Na_2SO_4) by species (*Helianthus paradoxus* and *Helianthus annuus*) and (b) ionic strength (0.00–0.51) by species. A three-way ANOVA determined there were significant differences ($P < 0.0001$) between salt treatments and ionic strengths for each species. Different letters indicate significant differences between means (Duncan's Multiple Range Test). Lines above the bars represent + one SD (standard deviation).

however, dry mass of *H. annuus* was significantly lower at an ionic strength of 0.09 (Fig. 1b, species × ionic strength interaction). The salt × ionic strength interaction has not been presented because the results can be seen within the three way interaction figure. The significant three-way interaction indicated that dry mass was dependent on species, salt type, and ionic strength (Table 2, Fig. 2a, b). *Helianthus paradoxus* dry mass was higher in Na_2SO_4 compared to NaCl (Fig. 2a), with the greatest dry mass at the mid ionic strengths (Fig. 2a). On the other hand, *H. annuus* dry mass was lower than *H. paradoxus*, but *H. annuus* did produce more dry mass in Na_2SO_4 compared to NaCl (Fig. 2b). In addition, as the ionic strength increased dry mass of *H. annuus* decreased.

In comparison to the Na_2SO_4 treatments, dry mass of both species was significantly reduced in NaCl at elevated ionic strengths (Duncan's Multiple Range Test, $P < 0.0001$, Fig. 2a, b). In the no-salt treatment, growth of *H. annuus* was elevated compared to *H. paradoxus* (not significantly). However, for all levels of salt addition, total dry mass of *H. paradoxus* was greater than *H. annuus*, yet the differences were salt dependent. For both species, mean total dry mass was elevated in Na_2SO_4 at an ionic strength of 0.09 compared to NaCl. Dry mass of *H. paradoxus* was 32% greater than dry mass of *H. annuus* in this treatment. In the 0.17 to 0.51 ionic strengths of Na_2SO_4, mean total dry mass of *H. paradoxus* was 2- to 12-fold greater than *H. annuus*. Both species produced less dry mass in NaCl treatments; however, *H. annuus* dry mass was reduced most by NaCl. Total dry mass of *H. paradoxus* was 7-fold greater than *H. annuus* in the 0.09 NaCl treatment. This difference between species in NaCl increased at the 0.17 and 0.34 ionic strengths of NaCl where dry mass of *H. paradoxus* was 15-fold greater than *H. annuus*.

DISCUSSION

Salt tolerance is the ability of a species to grow and adjust to the presence of a specific ion (ionic effect) or to adapt to the general effects of low water potentials (osmotic effect) (Ungar 1991). In this study, a potential ionic effect and an osmotic effect were investigated using NaCl and Na_2SO_4 salts at increasing ionic strengths. Both salts are found in *H. paradoxus* habitats at various concentrations (Boghici 1997; Van Auken and Bush 1998; Lexer et al. 2003). To differentiate between the effects of the two salts and the Cl^- and SO_4^{2-} anions, equal ionic strengths were used in the separate salt treatments (Barrow 1966). Due to greater salt tolerance of *H. annuus* in NaCl and mixed salt environments compared to *H. petiolaris* (both purported parental species) (Ashraf and Tufail 1995; Welch and Rieseberg 2002; Bush and Van Auken 2004; Karrenberg et al. 2006; DiCaterina et al. 2007), *H. annuus* was used as a comparative species for salt effects on growth of *H. paradoxus*.

As in previous studies, this investigation demonstrated that *H. paradoxus* was more salt tolerant than one of its parental species, *H. annuus*, and even produced slightly more dry mass in low saline soils compared to *H. annuus* (Figs. 1b, 2a, and 2b). *Helianthus paradoxus* produced 70% more dry mass than *H. annuus* over all treatments (data not shown) and consistently produced more dry mass in elevated salinity soils (Fig. 2a, b), especially in the sulfate treatments. Results of this greenhouse study are consistent with observations that *H. paradoxus* has greater fitness in saline conditions than *H.*

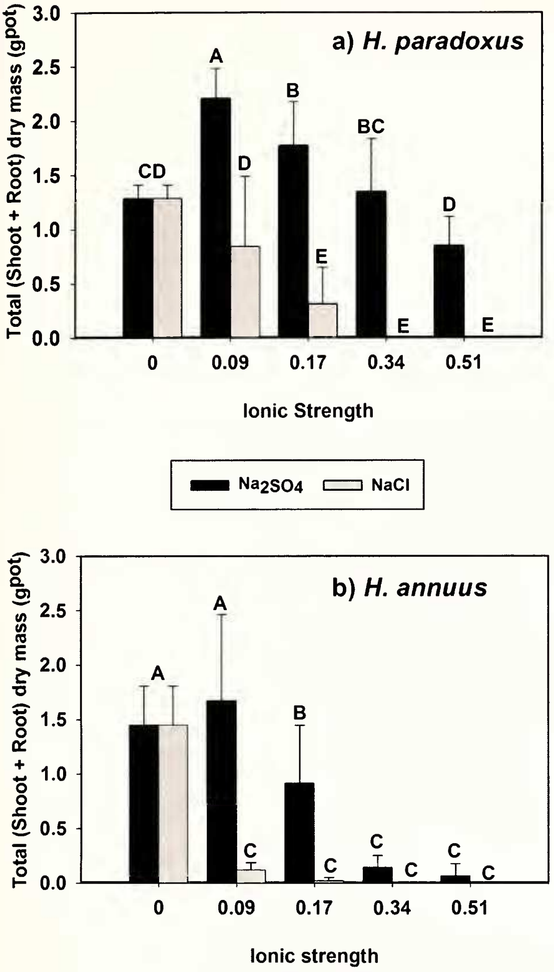

FIG. 2. Three-way interaction plots of total dry mass (bars) as the response variable for (a) *Helianthus paradoxus* and (b) *Helianthus annuus*. Salts were Na_2SO_4 (black bar) and NaCl (gray bar) at ionic strengths of 0.00–0.51. A three-way ANOVA determined there were significant differences ($P < 0.0001$) between salt treatments and ionic strengths for each species. Different letters indicate significant differences between means (Duncan's Multiple Range Test) within a species. Lines above the bars represent + one SD (standard deviation).

annuus (Welch and Rieseberg 2002; Bush and Van Auken 2004; Karrenberg et al. 2006), and that *H. annuus* is not expected to be found in areas with elevated soil salinity where *H. paradoxus* is able to grow and outcompete *H. annuus* and probably other non-halophytic species (Abbot 2003; Van Auken and Bush 2006).

Both *Helianthus* spp. exhibited a specific ionic growth inhibition at elevated levels of Cl^-, compared to SO_4^{2-}, and differences in salt tolerance between species were evident. For both species, NaCl caused plant mortality at ionic strengths of 0.34 and 0.51, yet *H. paradoxus* was more tolerant than *H. annuus* to low to moderate NaCl concentrations (Fig. 2). Greater dry mass production and survivorship of *H. paradoxus* plants compared to *H. annuus* in NaCl treatments was also observed by Welch and Rieseberg (2002) in corresponding NaCl treatments (100 and 200 mmol L^{-1} [~6 and 12 g kg^{-1}] NaCl). Plant survivorship of *H. annuus* indicated that low Na_2SO_4 levels seem to be less inhibitive than NaCl but significant dry mass reduction was still observed above 0.09 ionic strength treatments. Dry mass of *H. paradoxus*, on the other hand, was enhanced by low to moderate levels of Na_2SO_4 with 38 to 72% greater dry mass, relative to the no-salt treatment. This corresponds to field observations where *H. paradoxus* was most abundant in soil salinities (mainly Na, K, Ca, and Mg chlorides with less SO_4) ranging from 5 to 12 g kg^{-1} in the Diamond-Y Spring Preserve (Boghici 1997; Van Auken and Bush 1998; Bush 2006b; Grunstra and Van Auken 2007a, b).

Although previous studies did not differentiate between the effects of NaCl and Na_2SO_4 (Welch and Rieseberg 2002; Bush and Van Auken 2004; Karrenberg et al. 2006; Van Auken and Bush 2006), those studies support the salt tolerance of *H. paradoxus* to low concentrations of NaCl. Results consistently demonstrated that biomass of *H. annuus* was statistically reduced by NaCl alone (Welch and Rieseberg 2002) or by low levels of mixed salts (Na^+, Cl^-, and SO_4^{2-} included; Bush and Van Auken 2004; Karrenberg et al. 2006), while *H. paradoxus* demonstrated lower productivity in NaCl alone and greater growth and productivity in the presence of SO_4^{2-} as observed in the present study. Greater salt tolerance of *H. paradoxus* to NaCl, as compared to *H. annuus*, has been attributed to significantly greater fitness along with Na^+ accumulation, leaf succulence, and water use efficiency (Welch and Rieseberg 2002). Because the salts were at equal ionic strengths and Na^+ seems to serve as an osmoticum for *H. paradoxus* (Welch and Rieseberg 2002), it can be assumed that Cl^- is causing reduced productivity in comparison to SO_4^{2-}. Molar concentrations of Cl^- in the soil water of the Diamond-Y Spring Preserve are approximately 1.5 times that of molar concentrations of SO_4^{2-}; therefore, Cl- has the potential of inhibiting growth of *H. paradoxus* in its salt marsh habitat (Boghici 1997). However, these data in conjunction with previous salt studies (Welch and Rieseberg 2002; Bush and Van Auken 2004; Karrenberg et al. 2006) indicate that the presence of soil sulfate may have played an important role in the selection for and adaptation of *H. paradoxus* to the Na-Cl^--SO_4^{2-} rich environments. Further, poor tolerance to chloride and sulfate by *H. annuus* has limited its establishment and therefore, fitness in the *H. paradoxus* salt marsh habitat.

It should be noted that in order to maintain equal osmotic potentials between treatments, molar concentrations of Na^+ in NaCl treatments were 3.0 times that of Na_2SO_4 treatments. Nevertheless, the molar equivalents of Na^+ at 0.09 and 0.17 ionic strengths were between that of the 0.34 and 0.51 ionic strengths of Na_2SO_4 where dry mass production of *H. paradoxus* was not inhibited. Since an inert osmotic medium such as polyethylene glycol was not examined, an osmotic effect in combination with an ionic effect cannot be excluded (Katembe et al. 1998; Munns and Tester 2008).

In the limited studies comparing phytotoxicity of both salts, greater toxicity to NaCl compared to Na_2SO_4 has been demonstrated for other salt tolerant glycophytes and halophytes (Manchanda et al. 1982; Curtin et al. 1993; Franklin and Zwiazek 2004; Pagter et al. 2009). Chloride is more toxic to plants than sulfate possibly due to synergistic phytotoxicity effects with Na^+, differential inhibition of enzyme activity, reduction in plant productivity, and imbalance of nutritional status (Greenway and Munns 1980; Manchanda et al. 1982; Curtin et al. 1993; Wang et al. 1997; Veira Dos Santos and Caldeira 1999; Franklin and Zwiazek 2004). Ion toxicity is dependent on whether the plant possesses adaptations to tolerate the osmotic stress and to exclude and/or compartmentalize the ion. Although not yet documented, it is possible that *H. paradoxus* accumulated Cl^- along with Na^+ and may even be more sensitive to Cl^- compared to SO_4^{2-} due to poor compartmentalization into vacuoles (Greenway and Munns 1980; Flowers et al. 1986; Munns 1993; Rajakaruna et al. 2003). Chloride may be considered more toxic sometimes because of poor salt tolerance response and thus, high accumulation of Cl^- over Na^+, or in this case SO_4^{2-}. Toxic cytoplasmic Cl^- concentrations have not yet been determined but are assumed to be equal to or slightly lower than Na^+ (Flowers et al. 1986; Greenway and Munns 1980; Munns and Tester 2008).

Sulfate may be required for salt tolerance in *H. paradoxus*. Tissue ion concentrations were not examined in this study, but previous work suggested that (as in other halophytes) SO_4^{2-}, along with Na^+, may be an important vacuolar osmoticum in plant tissue (Greenway and Munns 1980; Karrenberg et al. 2006; Johnston 2006). Leaf sodium and sulfur concentrations were shown to be inversely related to calcium, magnesium, and potassium concentrations but positively related to biomass and succulence in *H. paradoxus* (Karrenberg et al. 2006). Interestingly, several studies have found a correlation between plants inhabiting waterlogged sulfate rich soils and the presence of vacuole stored flavonoid sulfates which may serve to detoxify excess sulfates alone or in combination with sodium (Harborne 1975; Tomas-Barberan et al. 1987; Rajakaruna et al. 2003). A possible role of sulphur in the salt tolerance of halophytes also includes the production of methylated sulfonium compounds that accumulate in the cytosol as osmotically compatible organic solutes for compartmentalization of Na^+ and Cl^- in vacuoles. These organic solutes also serve to detoxify sulfides in salt marsh plants (Flowers and Colmer 2008). Flavonoid sulfates or methylated sulfonium compounds have not been measured in tissues of *H. paradoxus*; however, Na_2SO_4 tolerance of this species together with Na^+ and SO_4^{2-} accumulation suggests a possible detoxification mechanism not yet identified.

Ecological isolation of *H. paradoxus* within the inland salt marshes of west Texas and New Mexico may in part depend on the elevated levels of soil salinity found in soils of these habitats (Boghici 1997; McDonald 1999; Van Auken and Bush 1998, 2006; Abbott 2003). Halophytes are limited to saline environments because of an advantageous adaptation to excess salts and a reduction of competitive ability in non-saline environments (Ungar 1991). Distribution of *H. paradoxus* plants appears to be dependent on low to moderate soil salinity levels at the Diamond-Y Spring Preserve where Na^+, Cl^- and SO_4^{2-} are the prevalent salts from groundwater discharge, while Ca^{2+}, Mg^{2+}, and HCO_3^- are secondary ions (Boghici 1997; Van Auken and Bush 1998; Bush 2006b; Grunstra and Van Auken 2007a, b). In the present study, *H. paradoxus* outperformed *H. annuus* in both soil salt treatments, partially explaining the inability of *H. annuus* to survive the salt marsh habitat of *H. paradoxus* (Lexer et al. 2003; Bush and Van Auken 2004). The presence of salts excluded *H. annuus* from colonizing the salt marsh (Abbott 2003; Lexer et al. 2003), while the salt tolerance of *H. paradoxus* to both NaCl and Na_2SO_4 has allowed this species to establish in the Diamond-Y Spring Preserve and other salt marsh environments in west Texas and New Mexico. Further, tolerance to high concentrations of Na_2SO_4, as demonstrated by significantly greater productivity relative to non-saline conditions, suggests that *H. paradoxus* may experience a physiological stress response without elevated Na_2SO_4, which is necessary for optimum growth and perhaps necessary for salt tolerance (Munns and Tester 2008). The establishment of *H. paradoxus* in the Diamond-Y Spring Preserve has been promoted by the selection for transgressive phenotypes promoting salt tolerance (sodium exclusion, calcium uptake, and leaf succulence), as demonstrated by *H. paradoxus*, along with the presence of specific ions (Na^+, Ca^{2+}, and SO_4^{2-}) in the salt marsh habitat (Abbott 2003; Lexer et al. 2003; Karrenberg et al. 2006). In addition, both parental species are poor competitors in field-like elevated soil salinity conditions, while *H. paradoxus* is a poor competitor against *H. annuus* in nonsaline conditions (Bush and Van Auken 2004).

The potential for hybridization is still present and environmental conditions isolating hybrid *Helianthus* spp. from parental genotypes appears to have been an important factor in their adaptive evolution of greater fitness in their respective habitats (Lexer et al. 2003; Whitney et al. 2010; Donovan et al. 2010). Populations of *H. annuus* are found in disturbed pockets of isolated deep, nonsaline soil. *Helianthus petiolaris*, the other reported parent species to *H. paradoxus* is also found in some isolated, low saline, sandy soils. However, environmental factors such as the soil conditions required for the isolation and survival of some of the *H. annuus* × *H. petiolaris* hybrids was and continues to be the saline soils surrounding the isolated springs in this area of west Texas and New Mexico (Abbott 2003; Lexer et al. 2003). The unique salt tolerance of *H. paradoxus* compared to its parental species along with establishment in the Na^+-Cl^--SO_4^{2-} dominated salt marshes will continue to promote the ecological isolation of *H. paradoxus*.

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